



Update on the implication of potassium channels in autism: K⁺ channel autism spectrum disorder

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Autism spectrum disorders (ASDs) are characterized by impaired ability to properly implement environmental stimuli that are essential to achieve a state of social and cultural exchange. Indeed, the main features of ASD are impairments of interpersonal relationships, verbal and non-verbal communication and restricted and repetitive behaviors. These aspects are often accompanied by several comorbidities such as motor delay, praxis impairment, gait abnormalities, insomnia, and above all epilepsy. Genetic analyses of autistic individuals uncovered deleterious mutations in several K⁺ channel types strengthening the notion that their intrinsic dysfunction may play a central etiologic role in ASD. However, indirect implication of K⁺ channels in ASD has been also reported. For instance, loss of *fragile X mental retardation protein* (FMRP) results in K⁺ channels deregulation, network dysfunction and ASD-like cognitive and behavioral symptoms. This review provides an update on direct and indirect implications of K⁺ channels in ASDs. Owing to a mounting body of evidence associating a channelopathy pathogenesis to autism and showing that nearly 500 ion channel proteins are encoded by the human genome, we propose to classify ASDs - whose susceptibility is significantly enhanced by ion channels defects, either in a monogenic or multigenic condition - in a new category named “**channelAutism Spectrum Disorder**” (*channelASD*; *cASD*) and introduce a new taxonomy (e.g., K_vx.y-*channelASD* and likewise Na_vx.y-*channelASD*, Ca_vx.y-*channelASD*; etc.). This review also highlights some degree of clinical and genetic overlap between K⁺ channelASDs and K⁺ channelepsies, whereby such correlation suggests that a subcategory characterized by a *channelASD-channelepsy phenotype* may be distinguished. Ultimately, this overview aims to further understand the different clinical subgroups and help parse out the distinct biological basis of autism that are essential to establish patient-tailored treatments.

Keywords: K⁺ channels, Kv4.2 (*KCND2*), Kv7.3 (*KCNQ3*), K_{Ca}1.1 (*KCNMA1*), Kir2.1 (*KCNJ2*), Kir4.1 (*KCNJ10*), FMRP (*FMR1*), channelASD-channelepsy phenotype

INTRODUCTION

Nearly 70 million people worldwide suffer from ASD. 700,000 are from USA and the estimated cost to society of caring for these children has been \$11.5 billion in 2011 (Lavelle et al., 2014). ASD is a group of heterogeneous neurodevelopmental disorders previously dealt with as single pathologies: autistic disorder, pervasive developmental disorder-not otherwise specified (PDD-NOS), Asperger syndrome, childhood disintegrative disorder also known as Heller syndrome, and Rett syndrome. Specific impairments before age three, in each of the following three core domains fit the diagnostic criteria for ASD: **social** (difficulty in interacting with people, in reading facial expressions and making eye contact), **language** (difficulty in using or understanding language, tending to focus attention and conversation on a limited number of topics, frequently repeat phrases, and have very limited speech ability), and **restricted/repetitive behavior** (excessive attention to routines and difficulty in adjusting to new surroundings or changes in routine). Given the complexity of the disorder and the variety and

severity of the symptoms, ASD is thought to be caused by multiple factors interacting in a complex way. Genetic defects, both inherited or occurring spontaneously, undoubtedly play a role in increasing the susceptibility to the disorder by affecting brain development and proper neural networking. Neurological comorbidities are often observed in ASD patients and they are associated with more clinical severity. The most noted is epilepsy, comorbid in 30% of ASD, about 50 times higher than in the general population (Tuchman and Rapin, 2002; Mouridsen et al., 2011). Cognitive and behavioral deficits occurs in up to 46% of children with epilepsy (Clarke et al., 2005; Matsuo et al., 2010) suggesting that, at least in these cases, the excitability imbalance could be a common denominator. Another comorbidity observed in nearly 90% of genetic syndromes associated with ASDs is motor delay. Delays occur in both gross and fine motor domains, while deficits are documented in praxis, motor planning, gait, coordination, and postural control (Rinehart and McGinley, 2010; Van Waelvelde et al., 2010). Several lines of evidence suggest that motor delay is

caused by aberrant neural circuitry mostly in the cerebellum and fronto-striatal regions (Rinehart et al., 2006; Wegiel et al., 2013).

The relative risk for a child to develop the disease is increased ~25-fold in families in which a sibling is affected (Jorde et al., 1991). Unfortunately, the genetic etiology of autism is characterized by high locus heterogeneity, where *de novo* and rare inherited CNVs and SNV mutations in conjunction contribute to the overall genetic risk to develop the disease. To date, we know that defined mutations, genetic syndromes and *de novo* CNVs account for about 30% of ASD cases (Schaaf and Zoghbi, 2011). Linkage analyses of ASD patients have allowed the identification of *loss-* or *gain-of-function* mutations and chromosomal alterations in genes coding for several channel types, including K⁺ channels. These proteins are highly heterogenic and widely expressed in the CNS where they set the resting membrane potential of neurons and glia, shape action potentials and regulate firing, neurotransmitter release and wiring. At network level, K⁺ channels are involved in post-synaptic EPSP-spike coupling, activity dependent short- and long-term synaptic plasticity and information processing. Thus, these channels provide neurons, glia and CNS networks with distinct electrical identity and their dysfunction is a major contributing factor in excitability disequilibrium and network impairment. Animal models, in which these genes are deleted or mutated, often show epilepsy (Eijkelkamp et al., 2012; D'Adamo et al., 2013, 2014), motor impairment, learning and behavioral phenotypes that resemble somehow ASD. The insights, provided by investigations with these animal models, are valuable to understand neural networks abnormalities potentially underlying ASD. The plethora of ion channel types expressed by neurons and the numerous variations discovered in their relevant genes often generate confusion in the classification of the associated diseases. To further understand the clinical subgroups of autism, help parse out distinct biological basis of autism and identify tailored treatments, we propose to name the ion channels defects contributing significantly to either monogenic or multigenic ASDs “channelASD” and suggest a new taxonomy (e.g., K_vx.y-channelASD and likewise Na_vx.y-channelASD; Ca_vx.y-channelASD; etc.), according to a previously proposed nomenclature for ion channels dysfunctions underlying epilepsy (D'Adamo et al., 2013). Interestingly, this review will also show that there is a high degree of clinical overlap between channelASD and channelepsy. This strong correlation between a distinct subgroup of patients prompt us to also define a new phenotype: “channelASD-channelepsy,” according with our previously and presently introduced nomenclature.

VOLTAGE-GATED K⁺ CHANNELS

The first voltage-gated K⁺ channel (Kv) was cloned from the *Shaker* mutant of *Drosophila melanogaster* (Tempel et al., 1987). Since then, a number of other genes encoding for Kv channels have been identified from many different species. Based on sequence relatedness, Kv channels have been classified in subfamilies from Kv1 to Kv12 (Chandy and Gutman, 1993). The α subunit contains six transmembrane segments with the N- and C-termini residing inside the cell. The full crystal structure, provided for a Kv channel, confirmed that this channel is composed of four homologous pore-forming α subunits. These channels may exist either

as homomers, whenever four identical α -subunits are assembled or as heteromers, whenever different types of α -subunits heteropolymerize to form channels with distinct functional and pharmacological properties (Pessia, 2004).

Kv4.2 – ChannelASD

KCND2 gene encodes for the α subunit of the voltage-gated K⁺ channel Kv4.2 that generates an “A-type” current (I_A). When assembled in homotetramers, Kv4.2 channels activate at sub-threshold membrane potentials, inactivate rapidly, and quickly recover from inactivation (Zhu et al., 1999; Birnbaum et al., 2004). The availability of this channel type, in response to membrane potential fluctuation, is regulated by its peculiar inactivation kinetics occurring from the open state by a fast N-type mechanism or (*much more frequently*) from pre-open closed states, when the voltage-sensor domains activate but the pore fails to open (Bähring et al., 2001; Gebauer et al., 2004; Jerng et al., 2004; Barghaan and Bähring, 2009; Bähring and Covarrubias, 2011). *In vivo*, other proteins further tune-up Kv4.2 inactivation kinetics: the K⁺ channel interacting protein (KChIP) and the dipeptidyl-peptidase-like protein (DPP) that respectively inhibits and accentuates N-type open state inactivation (Wang et al., 2002; Jerng et al., 2005, 2009; Covarrubias et al., 2008). This channel type is mainly expressed in the hippocampal CA1 pyramidal neurons where it regulates the threshold for action potential initiation and repolarization, frequency-dependent AP broadening, and back-propagation of action potentials (Chen et al., 2006; Kim et al., 2007; Nerbonne et al., 2008). These notions were further corroborated by the evidence that Kv4.2 channel deletion lowers and increases the threshold for LTP and LTD induction, respectively (Chen et al., 2006; Zhao et al., 2011). Conversely, enhancement of Kv4.2 expression prevents LTP induction (Jung et al., 2008). The presence of Kv4.2 channels in hippocampus appears fundamental, mostly at early developmental stage when neuronal activity drives synaptic maturation and network refinement. At hippocampal synapses, the gradual reduction in GluN2B/GluN2A subunit ratio, during post-natal development, is correlated with AMPA expression and synaptic maturation. Ablation of Kv4.2 in mice abolished this phenomenon and resulted in a higher number of silent synapses in the adulthood (Kim and Hoffman, 2012). Noteworthy, both NMDA subunit composition and synaptic inactivity are rescued by reentering the channel (Kim and Hoffman, 2012).

Given the importance of Kv4.2 in brain development and functioning, defects of this channel have been unsurprisingly correlated with a broad spectrum of neurological disorders. Gene deletion in mice leads to increased susceptibility to convulsant stimuli (Barnwell et al., 2009) and truncating mutation of Kv4.2 in humans leads to temporal lobe epilepsy (Singh et al., 2006). Moreover, rare variants in KCND2 have been identified in individuals with autism, namely: a submicroscopic *de novo* deletion (Okamoto et al., 2011), three translocation breakpoints (at 7q22.1, 7q31.2 and 7q31.3) potentially deleterious (Scherer et al., 2003), and three substitution variants, (N544S; F538S; R539L) reported in three independent cases of autism (Mikhailov et al., 2008). Recently, exome sequencing of two identical twins affected by autism, with frequent and uncontrollable seizures, revealed a *de novo* heterozygous variant

in *KCND2* gene that resulted in the substitution V404M (Lee et al., 2014). Expression and functional characterization of Kv4.2 V404M channels in *Xenopus laevis* oocytes showed significantly slower inactivation than wild-type, leading to greater channel availability in response to depolarization. These functional alterations persisted when the mutated subunit was co-expressed with the wild-type (in 1:1 ratio to mimic the heterozygous nature of the variation), suggesting a dominant effect. The V404M impact on channel kinetics was attributed to impaired closed-state inactivation, because the effect was still evident in the presence of the auxiliary subunits KChIP3a or DPP10a (Lee et al., 2014).

Kv4.2 – ChannelASD linked to Fragile X syndrome

Kv4.2 channel expression may also participate in establishing the conditions for the development of ASDs, given that Kv4.2 mRNA can bind to the *fragile X mental retardation protein* (FMRP), which is associated to *fragile X syndrome* (FXS), the most common monogenic cause of autism and inherited intellectual retardation (Garber et al., 2008) often accompanied by seizures and poor motor coordination (Garber et al., 2008). FXS is present in ~5% of ASDs cases, and is the most likely cause of autism in those individuals. FXS is caused by an expansion of CGG triplets (>200) in the 5' untranslated region of the fragile X mental retardation gene 1 (*FMR1*), located on chromosome X (Krawczun et al., 1985; Fu et al., 1991), which results in the failure to produce the FMRP, required for normal neural development (Pieretti et al., 1991; Verheij et al., 1993; Santoro et al., 2012). This protein is predominantly post-synaptic, where is associated with ribosomal complexes and represses or promotes the translation of specific mRNAs (Siomi et al., 1993; Todd and Malter, 2002; Zalfa et al., 2003; Weiler et al., 2004; Muddashetty et al., 2007; Bechara et al., 2009). The function and localization of this protein make it a key regulator of synaptic plasticity, especially for all those activity dependent processes that require the synthesis of new proteins. In particular, FMRP appears to play a critical role in the maintenance of LTD and LTP in the hippocampus (Weiler et al., 1997; Huber et al., 2000, 2002; Hou et al., 2006; Lauterborn et al., 2007; Michalon et al., 2012). In hippocampal dendrites, FMRP is associated with Kv4.2 mRNA and prevents its translation leading to reduced channel expression at post-synaptic membrane (Lee et al., 2011). Consistent with this scenario, hippocampal neurons from *Fmr1*-null mice show higher levels of Kv4.2 protein than WT and reduced LTP, when induced by threshold stimuli (Lee et al., 2011; **Table 2**). The application of heteropodatoxin (HpTx2), a spider toxin that specifically blocks Kv4.2 channels, restores LTP in hippocampal slices derived from *Fmr1*-null mice (Lee et al., 2011). It is therefore likely that deregulated expression of Kv4.2, resulting from FMRP-dependent abnormalities, may contribute significantly to FXS pathogenesis and generate the conditions for ASDs development.

Kv7.3 – ChannelASD

The K⁺ channel subunit Kv7.3 is encoded by the *KCNQ3* gene and is expressed in the hippocampus and cortex where it assembles predominantly with the homologous Kv7.2 to form “M” channels (Wang et al., 1998). Generation of functional M channels by heteropolymerization of Kv7.2 (*KCNQ2*) with Kv7.4 (*KCNQ4*) or

Kv7.5 (*KCNQ5*) subunits has also been described (Kubisch et al., 1999; Schroeder et al., 2000). This channel type was identified by Brown and Adams (1980) as the principal mediator of muscarine-induced depolarization. They are located in electrically critical regions of neuronal membrane, generate a typical sub-threshold K⁺ conductance and regulate the excitability of many types of neurons. In hippocampal pyramidal cells, the channel is found in the peri-somatic regions where it modulates EPSPs integration and set the threshold for EPSP-spike coupling (Shah et al., 2008). The expression of M channels is finely tuned during the development of early forms of neuronal synchrony. Indeed, in immature CA3 pyramidal cells, the “giant depolarizing potentials” (GDPs) exist thanks to the excitatory nature of GABA in synergy with M channel down-regulation. The progressive disappearance of GDPs, following the first postnatal week, is matched by enhancement of M current density (Safilina et al., 2008) and development of more organized forms of activity, such as theta and gamma rhythms (Buzsáki and Draguhn, 2004).

KCNQ3 and *KCNQ2* gene mutations segregate with various forms of Kv7.3/Kv7.2-channelopathies (Maljevic et al., 2008; D'Adamo et al., 2013) such as benign familial neonatal convulsion (BFNC) and rolandic epilepsy. Importantly, >20% of patients with rolandic epilepsy have cognitive deficits and >10% display abnormal behavioral (ADHD, anxiety, depression, and pervasive developmental disorder; Tovia et al., 2011). Furthermore, 40% of patients with BFNC show delayed psychomotor development or intellectual disability (Steinlein et al., 2007). Several *KCNQ3* mutations, associated with BFNC, reduce heteromeric M current amplitudes (Singh et al., 2003). Mice carrying the heterozygous G311V mutation in the channel's pore show a lower threshold for epileptogenesis. While, homozygous mice exhibit spontaneous tonic-clonic seizures associated with reduced M current amplitudes and increased deactivation kinetics in hippocampal CA1 pyramidal cells (Singh et al., 2008). Interestingly, a *de novo* interstitial deletion in the long arm of chromosome 8, encompassing *KCNQ3*, has been identified in two children with a broad spectrum of congenital abnormalities, psychomotor delay and convulsions (Verheij et al., 2009). Another study from Gilling et al. (2013) identified the *de novo* reciprocal translocation *t*(3;8)(q21, q24) truncating *KCNQ3* gene and the missense mutation P574S in distinct autistic patients. The *de novo* translocation was identified in a Danish boy affected by idiopathic periodic trembling since the age of 2 days that persisted for the first 5 weeks of life. In one case, the P574S variant, which substitutes a phylogenetically conserved residue in the C-terminal region of Kv7.3, was inherited from the mother who suffered from major depression. Surprisingly, none of the patients harboring this variant had histories of seizures. Functionally, the P574S variant significantly reduces K⁺ current amplitude in oocytes when co-expressed with Kv7.5 subunit but not with Kv7.2 or Kv7.4 subunits (Gilling et al., 2013; **Table 1**). Given the importance of M current in the development of neuronal identity and regulation of excitability, its impairment in immature neurons can depolarize membrane potential increasing cell excitability, facilitating the onset of seizures and delaying the definition of complex neuronal rhythms that possibly result in autistic phenotype.

Table 1 | Direct involvement of K⁺ channels in ASD.

| Gene, channel, neurophysiological role | KO/cKO phenotype | Epilepsy: genetic mutation or chromosomal aberration/phenotype | ASD: genetic mutation or chromosomal aberration/phenotype | Reference |
|--|---|---|--|--|
| KCND2/Kv4.2: | | | | |
| – Neuronal: regulates the threshold for action potential initiation and repolarization, frequency-dependent AP broadening, and back-propagation of AP. | – <i>Kcnd2</i> ^{-/-} : display increased susceptibility to convulsant stimulation. | – Truncating mutation of Kv4.2: temporal lobe epilepsy. | – V404M: <i>gain-of-function</i> : slows channel inactivation leading to greater channel availability in response to depolarization. Found in two identical twins affected by autism, with frequent and uncontrollable seizures. | Chen et al. (2006), Singh et al. (2006), Kim et al. (2007), Nerbonne et al. (2008), Barnwell et al. (2009), Lee et al. (2014) |
| KCNQ3/Kv7.3: | | | | |
| – Neuronal: generation of sub-threshold potassium current <i>I_M</i> which, in the hippocampus, modulates EPSPs integration and set the threshold for EPSP-spike coupling. | | – G311V pore mutation: benign neonatal epilepsy. – <i>De novo</i> interstitial deletion in the long arm of chromosome 8 encompassing <i>KCNQ3</i> : psychomotor delay and convulsions. | – <i>De novo</i> reciprocal translocation t (3; 8) (q21, q24) truncating <i>KCNQ3</i> . Found in one patient with delayed verbal and social development affected by idiopathic periodic trembling. – P574S: <i>loss-of-function</i> significantly reduces potassium current amplitude in <i>Xenopus laevis</i> oocytes when co-expressed with Kv7.5 subunit but not with Kv7.2 or Kv7.4 subunits. Found in three patients affected by autism. | Singh et al. (2003, 2008), Shah et al. (2008), Verheij et al. (2009), Gilling et al. (2013) |
| KCNMA1/KCa 1.1 | | | | |
| – Neuronal: negatively regulates Ca ²⁺ entry and provides a damping mechanism for excitatory signals. | – <i>KCa 1.1</i> ^{-/-} and <i>PNIs-KCa 1.1</i> ^{-/-} : display deficit in cerebellar learning, ataxia, abnormal locomotion and pronounced lack of coordination. | – <i>KCNMA1 gain-of-function</i> mutations: cause generalized epilepsy and paroxysmal dyskinesia. | – A138V: the substitution creates a cryptic splice donor site in the second exon. Found in one patient affected by epilepsy, impairments of social interactions and communications skills, lack of spoken language and poor communicating gestures. – <i>De novo</i> 9q23/10q22 translocation destructing <i>KCNMA1</i> : found in one patient affected by impairments of social interactions and communication skills, lack of spoken language and poor communicating gestures. | Lancaster and Nicoll (1987), Robitaille and Charlton (1992), Shao et al. (1999), Hu et al. (2001), Du et al. (2005), Laumonier et al. (2006), Womack et al. (2009) |

(Continued)

Table 1 | Continued

| Gene, channel, neurophysiological role | KO/cKO phenotype | Epilepsy: genetic mutation or chromosomal aberration/phenotype | ASD: genetic mutation or chromosomal aberration/phenotype | Reference |
|---|---|---|---|--|
| KCNJ2/Kir2.1 – Glial: in combination with Kir4.1 controls astrocyte-mediated K ⁺ buffering. – Neuronal: plays an important role in DGCs firing properties during development. | | – <i>KCNJ2</i> loss-of-function mutation: cause Andersen-Tawil syndrome a condition characterized by long QT-syndrome, cardiac arrhythmia, skeletal abnormalities, mood disorders and seizures. | – K346T: <i>gain-of-function</i> : leads to (i) enhanced the channel's stability at the plasma membrane; (ii) reduced protein ubiquitylation and degradation; (iii) altered protein compartmentalization in lipid rafts, by targeting more channels to cholesterol-poor domains; (iv) reduced interactions with caveolin 2. Found in two identical twins affected by epilepsy, impaired social interaction, absence of speech, repetitive behaviors and intellectual disability. | Bordey and Sontheimer (1998), Haruna et al. (2007), Jabs et al. (2008), Chan et al. (2010), Ambrosini et al. (2014) |
| KCNJ10/Kir4.1 – Glial: potassium buffering, astrocytes and oligodendrocytes differentiation. – Neuronal: (regulates) expressed in <i>locus coeruleus</i> neurons | – <i>Kcnj10</i> ^{-/-} : displays severe motor deficits, ipomyelination in the spinal cord, severe spongiform vacuolation and damaged astrocytes. – cKO <i>gfa2</i> directed: displays pronounced body tremor, lethargy and ataxia as well as visual placing deficiency and stress induced seizures. | – <i>KCNJ10</i> loss-of-function mutations: cause EAST or SeSAME syndrome. | – R18Q: <i>gain-of-function</i> , leads to increased channel surface expression. Found in two identical twins affected by epilepsy, impaired social interaction, absence of speech, repetitive behaviors and intellectual disability. – V84M: <i>gain-of-function</i> , leads to increase channel unit conductance compared to WT. Found in one patient affected by poor social gaze, no response to name, absence of language development and seizures. | Orkand et al. (1966), Neusch et al. (2001), Djukic et al. (2007), Higashimori and Sontheimer (2007), Bockenhauer et al. (2009), Scholl et al. (2009), Reichold et al. (2010), Sicca et al. (2011), Sibille et al. (2014) |

Ca²⁺-ACTIVATED K⁺ CHANNELS

The calcium-activated K⁺ (K_{Ca}) channels are highly conserved across species, and widely expressed in the human brain. The phylogenetic tree of K_{Ca} channels shows that they are made of two well distinct groups (Wei et al., 2005), the large conductance (BK; K_{Ca}1.1), and the small/intermediate-conductance (SK/IK; K_{Ca}2.1, K_{Ca}2.2, K_{Ca}2.3, K_{Ca}3.1) K_{Ca} channels. With regard to gating mechanism, the Ca²⁺ sensitivity of SK/IK channels is provided by tightly bound calmodulin (Xia et al., 1998; Fanger et al., 1999), in contrast to the direct binding of Ca²⁺ at specific internal sites on the channel protein of K_{Ca}1.1 channels (Lee and Cui, 2010). Moreover, unlike the SK/IK channels, K_{Ca}1.1 channels are also activated by voltage. In brain neurons, K_{Ca} channels are widely distributed in axons and presynaptic terminals (Knaus et al., 1996; Blank et al., 2004), often located close to voltage-gated Ca²⁺ channels Ca_v (Marrion and Tavalin, 1998). Ca²⁺ influx that results from neuronal excitation activates K_{Ca} channels, whose outward K⁺ flux contributes to terminate the action potential, establish the afterhyperpolarization (AHP) and close Ca_v channels. This negative feed-back control has been generally assumed to make K_{Ca} channels critical players in opposing repetitive firing and hyperexcitability typical of epileptic disorders. To date mutations only in the K_{Ca}1.1 channel type have been clearly associated to both autism and epilepsy.

K_{Ca}1.1 – Channel/ASD

The *KCNMA1* gene encodes for the α subunit of K_{Ca}1.1 channel which possesses a tetrameric structure with four optional auxiliary β subunits (Sausbier et al., 2004). Channel activation requires both an increase in intracellular Ca²⁺ concentration and membrane depolarization (Storm, 1987; Robitaille et al., 1993). Thanks to its Ca²⁺- and voltage-dependence it negatively regulates Ca²⁺ entry and provides a damping mechanism for excitatory signals in many neuronal types. K_{Ca}1.1 channels are expressed in the hippocampus, cortex and cerebellum where they contribute to both action potential repolarization and brief AHP (Lancaster and Nicoll, 1987; Robitaille and Charlton, 1992; Shao et al., 1999; Hu et al., 2001; Womack et al., 2009). Experimentally, the contribution of presynaptic I_{KCa1.1} is not easily detectable under basal conditions (Storm, 1987). It can be unmasked by blocking voltage-gated K⁺ channels with 4-aminopyridine that broadens the presynaptic action potential. Under these conditions, blocking K_{Ca}1.1 channels in CA1 with *iberiotoxin* (IbTX) causes further broadening of the presynaptic compound action potential, enhancement of synaptic transmission and reduced paired-pulse facilitation ratio (Hu et al., 2001). In cerebellar Purkinje neurons (PNs), the contribution of K_{Ca}1.1 channel to the AHP phase is developmentally regulated with a greater contribution in immature neurons than in adult PNs (Womack et al., 2009). *In vivo*, deletion of the *Kcnma1* gene profoundly alters cerebellar function: K_{Ca}1.1^{-/-} mice show an abnormal conditioned eye blink response, abnormal locomotion and pronounced lack of coordination (Sausbier et al., 2004). These changes are, in part, the direct consequence of a profound disinhibition of deep cerebellar nuclei (DCN). Indeed, while all the wild-type PNs discharge spontaneous APs at baseline, 50% of K_{Ca}1.1^{-/-} PNs become

quiescent. Since these silent neurons have a depolarized membrane potential, a depolarization block mediated by inactivated voltage-gated Na⁺ channels has been invoked as the underlying molecular mechanism (Sausbier et al., 2004). Thus, K_{Ca}1.1 channels ablation dramatically lowers the overall discharge activity of PNs. The close correlation between the loss of the K_{Ca}1.1 channel in PNs and the cerebellar deficit was further confirmed by the genesis of PN-K_{Ca}1.1^{-/-} mice, where K_{Ca}1.1 expression was specifically abolished in PNs (Chen et al., 2010). These animals recapitulate the motor deficits observed in K_{Ca}1.1^{-/-} mice, although to a lesser extent and show, *in vivo*, a reduction of both simple spike (SS) and complex spike (CS) activity in PNs. Recall that SS is generated by afferent parallel fibers (PF) while CS is induced by climbing fibers (CF). In these mice, the olivo-cerebellar circuit is likely deregulated since reductions in PNs inhibitory inputs result in an enhancement of DCNs activity which, in turn, exert an excessive inhibition on downstream inferior olive (IO). Interestingly, the application of muscimol (a GABA_A receptor agonist) onto DCN partially restores CS activity (Chen et al., 2010).

In humans, the *KCNMA1* gain-of-function mutation D434G has been found in patients suffering from K_{Ca}1.1-channelopathy that is characterized by generalized epilepsy and paroxysmal dyskinesia (Du et al., 2005; D'Adamo et al., 2013). Expression studies indicated that the D434G mutant channel results in markedly greater macroscopic currents and single-channel open probability due to ~5-fold increase in Ca²⁺ sensitivity (Du et al., 2005). The enhancement of K_{Ca}1.1 channel activity may lead to increased excitability by inducing rapid repolarization of action potentials, thus allowing neurons to fire at faster rates (Du et al., 2005). The possible role of K_{Ca}1.1 channels in ASD phenotype has been highlighted by Laumonnier et al. (2006) who have identified both the substitution A138V and the *de novo* translocation t(9;10)(q23;q22) in the *KCNMA1* gene of two unrelated autistic patients (Table 1). The autistic patient carrying the substitution displayed epilepsy, impairments of reciprocal social interactions and communications skills, lack of spoken language and poor communicating gestures. The A138V substitution is located within the second exon of *KCNMA1* and changes a residue highly conserved in evolution from *C. elegans* to mammals. Sequence analysis showed that the substitution creates a cryptic splice donor site that likely impairs channel expression. The *de novo* translocation was found in a 6 year old boy, displaying typical symptoms of autism. The translocation separates the promoter of *KCNMA1* from the rest of the gene, leading to a non-functional allele and haploinsufficiency. In fact, the *KCNMA1* transcript levels (determined from patient's lymphoblastoid cells) were halved. In addition, patch-clamp recordings from these cells revealed depolarized V_m, increased input resistance and halved IbTX-sensitive current (Laumonnier et al., 2006). Physiological K_{Ca}1.1 channel dosage is crucial for network functionality. Indeed, either loss or gain of K_{Ca}1.1 channel activity are both capable to unbalance cell excitability, markedly (Sausbier et al., 2004; Du et al., 2005; Chen et al., 2010). K_{Ca}1.1 loss-of-function mutations likely alter pyramidal neurons excitability and result in impairment of neural networks in hippocampus, an area implicated in cognition, mood disorders, and ASD. However,

Table 2 | Indirect involvement of K⁺ channels in ASD.

| Gene/channel | Neurophysiological role | Regulation by FMRP | Effect of FMRP loss at network level | Reference |
|-------------------------------------|--|---|---|---|
| KCND2/K_v4.2 | See: Table 1 . | – FMRP regulates K _v 4.2 mRNA translation. | – Higher K _v 4.2 expression in hippocampal neurons resulting in reduced LTP. | Kim and Hoffman (2008), Lee et al. (2011) |
| KCNMA1/K_{Ca}1.1(BK) | See: Table 1 . | – FMRP modulates Ca ²⁺ sensitivity of channels via interactions with the regulatory β4 subunits. | – Excessive AP broadening during repetitive activity, enhanced pre-synaptic Ca ²⁺ influx and abnormal increased STP. | Deng et al. (2011, 2013) |
| KCNT1/SLACK | – Neuronal: contributes to the slow hyperpolarization that follows repetitive firing and regulates the rate of bursting. | – FMRP binds to the <i>Slack</i> cytoplasmic C-terminal and induces an increase in channel opening. | – Reduced <i>I_{K(Na)}</i> component in MNTB neurons. | Bhattacharjee and Kaczmarek (2005), Brown et al. (2010) |

these mutations may also affect cerebellar PNs excitability, development, learning and memory processes, suggesting that K_{Ca}1.1 channels dysfunction may impact these crucial neurophysiological processes occurring within the cerebellum and result in the psychomotor development and cognition impairments of ASD (see below).

K_{Ca}1.1 – and SLACK – Channel/ASD linked to Fragile X syndrome

Recently, K_{Ca}1.1 channels have been implicated in ASD on a different ground, since their activity is regulated by FMRP, whose mutation produces FXS. We have mentioned previously that FMRP is mainly located postsynaptically, however, important pre-synaptic functions have been recently unmasked. FMRP is present in developing growth cones of axons (Antar et al., 2006), and it has been proposed to play pre-synaptic roles in neural wiring establishment (Hanson and Madison, 2007; Christie et al., 2009). Importantly, in pre-synaptic terminals FMRP regulates K_{Ca}1.1 channel Ca²⁺ sensitivity via interactions with the channel's regulatory β4 subunit (Deng et al., 2013). Loss of FMRP, as obtained in *Fmr1*-null mice, reduces K_{Ca}1.1 Ca²⁺ sensitivity leading to smaller current, AP broadening and increased short-term plasticity (STP) in hippocampal CA3 pyramidal neurons (Deng et al., 2011, 2013; **Table 2**). The application of IbTX, a selective inhibitor of K_{Ca}1.1 channels, reproduced in WT mice the effects observed in *Fmr1*-null mice (Deng et al., 2013). In addition, *Fmr1*-null mice displayed abnormal eye-blink conditioning, atypically elongated PNs spines and enhanced LTD at PF-PNs synapses, leading to reduced PNs response to PF stimuli. The enhancement in LTD induction found in *Fmr1*-null mice was reproduced in animals where the *Fmr1* gene was ablated specifically in PNs. This more direct evidence supports the notion that intrinsic PNs defects account for altered synaptic plasticity in the cerebellum of animals lacking FMRP and the crucial role that this brain structure may play in ASDs (Koekkoek et al., 2005).

Notably, FMRP can also bind to Na⁺-activated K⁺ channel *Slack*, and thus regulate its activity (Brown et al., 2010). This

channel type is mainly expressed in the cortex, hippocampus, olfactory bulb and lateral MNTB neurons of the brainstem (Brown et al., 2008) where it contributes to the slow hyperpolarization that follows repetitive firing and regulates the rate of bursting (Bhattacharjee and Kaczmarek, 2005). At the single channel level, *Slack* generates a very high conductance (~180 pS) with frequent transitions to a relatively long-lived sub-state with a conductance about one-third that of the fully open state. The binding of FMRP to the cytoplasmic C-terminal of *Slack* induces a fast and dramatic effect on these channel gating properties, namely, the transition to the sub-states is almost completely abolished, and there is a marked increase in channel opening (Brown et al., 2010). In *Fmr1*-KO mice, whole-cell recording of lateral MNTB neurons shows a significantly reduced *I_{K(Na)}* component compared to WT, consistent with a role for FMRP in regulating the activity of native *Slack* channels (Brown et al., 2010). Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is caused by mutations in the *CHRNA4*, *CHRNA2*, *CHRNA2*, or *KCNT1*(*Slack*) genes (Steinlein et al., 1995; De Fusco et al., 2000; Aridon et al., 2006; Heron et al., 2012). Interestingly, intellectual disability only occurs in those patients who carry mutations in *Slack* channels (Heron et al., 2012), further suggesting a role for this channel type in both epilepsy and cognitive disorders (reviewed in Kim and Kaczmarek, 2014).

INWARDLY RECTIFYING K⁺ CHANNELS

Inwardly rectifying K⁺ (Kir) channels take their name from the greater conductance at potentials negative to *E_K*, while at more positive values the outward flow of K⁺ ions is variably inhibited by cytoplasmic polyamines and Mg²⁺, by means of affinity dependent blockade. Indeed, the different sensitivity of Kir channels to polyamines and Mg²⁺ dictates the degree of rectification from weak to strong (Matsuda et al., 1987; Lopatin et al., 1994; Lu and Mac Kinnon, 1994; Stanfield et al., 1994). Cells that possess large Kir conductance display a resting membrane potential (*V_m*) close to *E_K*. Beside setting the *V_m* values, Kir channels of excitable cells play key roles in the regulation of action potential duration and excitability. Several Kir clones have been identified

and classified into seven major subfamilies, Kir1.x-Kir7.x (Bond et al., 1994; Hibino et al., 2010). Structurally, a Kir subunit possesses two transmembrane domains separated by a pore-forming region, serving as the “ion-selectivity filter,” with both the N- and C-termini residing inside the cell. Four subunits are assembled as both homotetramers and heterotetramers (Hibino et al., 2010).

Kir2.1 – ChannelASD

KCNJ2 encodes for Kir2.1 channels that possess a conductance of ~30 pS and generate macroscopic currents with strong rectification properties. High levels of *KCNJ2* transcript are found in the brain, heart and skeletal muscle. In the brain, Kir2.1 is predominantly expressed in the hippocampus, caudate, putamen, nucleus accumbens, and to lower levels in habenula and amygdala (Karschin et al., 1996), where it contributes to control neuronal excitability. In particular, the amplitude of Kir2.1 currents is small in young dentate granule neurons (DGCs) and increases ~3-fold in mature DGCs to optimize their excitability. Thus, Kir2.1 channels play an important role in DGCs firing properties during development (Mongiat et al., 2009). Kir2.1 is also expressed in astrocytes and, in combination with Kir4.1, controls astrocyte-mediated K⁺ buffering (*see below*; Bordey and Sontheimer, 1998; Jabs et al., 2008; Chever et al., 2010). *Loss-of-function* mutations in the *KCNJ2* gene are responsible for the rare Andersen-Tawil syndrome (OMIM 170390) a condition characterized by long QT-syndrome, cardiac arrhythmia, skeletal abnormalities, periodic paralysis, mood disorders, and seizures (Haruna et al., 2007; Chan et al., 2010). Conversely, Kir2.1 *gain-of-function* mutations segregate with SQT3 syndrome (OMIM 609622), another cardiac disorder characterized by QT shortening, ventricular tachyarrhythmias and atrial fibrillation. Indeed, in the heart Kir2.1 is a component of the inward-rectifier current IK1, which provides substantial repolarizing current during the terminal repolarization phase of the cardiac action potential and is the primary conductance controlling the diastolic membrane potential (Hutter and Noble, 1960; Sanguinetti and Tristani-Firouzi, 2000).

Recently, we reported on the identification of a new K346T heterozygous mutation in the *KCNJ2* of monozygotic twins displaying autism and epilepsy *in cis* with the previously detected R18Q variant in *KCNJ10* (Sicca et al., 2011; Ambrosini et al., 2014; **Table 1**). The twins also showed an electrocardiogram (ECG) with a markedly short repolarization time and conspicuously narrow and peaked T waves (QTc interval, 331 ms). K346T expression resulted in larger homozygous and heterozygous K⁺ currents due to increased surface expression of the channel in oocytes, HEK293 and glial-like cells. Functionally, several deleterious defects were also described for this novel *KCNJ2* variant which: (i) enhanced the channel's stability at the plasma membrane; (ii) reduced protein ubiquitylation and degradation; (iii) altered protein compartmentalization in lipid rafts, by targeting more channels to cholesterol-poor domains; (iv) reduced interactions with caveolin 2. All these molecular mechanisms contributed to causing Kir2.1 *gain-of-function*. Notably, the mutation promotes the surface expression of the channels particularly at end-feet, filopodia-like structures and cell-cell contacts. These structures are essential for astrocyte-mediated K⁺ siphoning through Kir2.1,

Kir4.1, and Kir5.1 channels, all of which could be influenced by the K346T mutation. Given that Kir2 channels also contribute to regulating neuronal excitability, cell differentiation, synaptic plasticity and wiring, their dysfunction may impact these crucial neurophysiological processes. Therefore, this study proposed that genetically induced Kir2.1 defects, beside causing SQT3 syndrome, may possibly result in functional impairment of neural networks where this channel type resides and contributes to ASDs pathogenesis (Ambrosini et al., 2014).

Kir4.1 – ChannelASD

The *KCNJ10* gene encodes for Kir4.1 channels (Bond et al., 1994), which forms homomeric channels or polymerize with Kir5.1 (*KCNJ16*) to form heterotetramers (Pessia et al., 1996) highly sensitive to pH (Tucker et al., 2000; Pessia et al., 2001; Casamassima et al., 2003; D'Adamo et al., 2011b). Kir4.1 is mainly expressed in *locus coeruleus* neurons, oligodendrocytes and astrocytes surrounding synapses and blood vessels from cortex, thalamus, hippocampus, and brainstem (Takumi et al., 1995; Higashi et al., 2001; D'Adamo et al., 2011b). In these cells, Kir4.1 channels generate high K⁺ permeability driving the membrane potential near E_K values. During excitatory synaptic activity, astrocytes surrounding synapses slowly depolarize because of K⁺ influx across their membranes (Orkand et al., 1966). This prolonged inward K⁺ current is mediated mainly by Kir4.1 and is synchronized with synaptic and spiking activity (Sibille et al., 2014). Thanks to the functional coupling between neurons and astrocytes the excess of extracellular K⁺ ions, resulting from intense neuronal firing, is taken up by astrocytes by flowing through Kir4.1 channels. Then, K⁺ ions are transferred toward sites where their concentration is kept at low levels by the gap-junction cell-synctium (Kuffler and Nicholls, 1966). By this mechanism, known as “K⁺ siphoning,” the concentration of extracellular K⁺ in CNS is strictly ruled about 2–3 mM, avoiding neuronal after-discharges and depolarization block. In the mouse hippocampus, Kir4.1 is significantly up-regulated between P3 and P12 (Seifert et al., 2009) and its expression is closely related to cell differentiation. In fact, Kir4.1 channels are absent in immature proliferating cells of the glia, and its progressive expression is linked to astrocytes hyperpolarization, differentiation and exit from cell cycle (from G2/M to G0/G1; Higashimori and Sontheimer, 2007). Genetic ablation of Kir4.1 in rodents results in severe motor deficits, hypomyelination in the spinal cord, severe spongiform vacuolation, astrocytes with immature morphology and premature death before P24 (Neusch et al., 2001). Conditional ablation of Kir4.1, exclusively in astrocytes, resulted in a mouse phenotype characterized by pronounced body tremor, lethargy, ataxia as well as visual placing deficiency and stress-induced seizures (Djukic et al., 2007). In humans, *KCNJ10 loss-of-function* mutations segregate with the EAST or SeSAME syndrome, a rare autosomic recessive disorder characterized by epilepsy, ataxia, sensorineural deafness, and tubulopathy. The patients develop tonic-clonic seizures in infancy followed by motor delay, speech and mental retardation (Bockenhauer et al., 2009; Scholl et al., 2009; Reichold et al., 2010). In line with the pathophysiological relationship between the Kir4.1 channel impairment and both

epilepsy and developmental disorders, we investigated the frequency of *KCNJ10* mutations in several children with cryptogenic epilepsy and autism spectrum traits. We found two inherited heterozygous *KCNJ10* mutations at residues highly conserved in mammalian and vertebrate orthologs: R18Q in two identical twins and V84M in another child. The expression of mutant channels in *Xenopus laevis* oocytes resulted in current/voltage relationships with greater amplitudes than WT for both mutations, suggesting a *gain-of-function* effect. Patch-clamp single-channel recordings revealed that the V84M mutation increased the unit conductance ~ 1.5 fold compared to WT. The R18Q mutation altered neither the unit conductance nor the single channel parameters, yet increased the channel surface expression. Clinically, the two identical twins showed impaired social interaction, sleep difficulties, hypotonia and both exhibited epileptic spasms occurring within the same 24 h period. Other symptoms, typical of ASD, included clumsiness, absence of speech, severe disorder of social interaction, stereotypies, repetitive behaviors, symptoms of anxiety, depression, obsessive compulsive disorder and intellectual disability (IQ: 58). The child harboring the V84M mutation showed normal psychomotor development until 12 months of age, when ASD symptoms such as poor social gaze, no response to name, absence of language development, and withdrawal behaviors became evident. At the age of six, he experienced complex partial seizures. EEG recordings showed synchronous and asynchronous paroxysmal abnormalities over frontal regions in both hemispheres, tending to spread (Sicca et al., 2011; **Table 1**). Co-occurrence of epilepsy and ASD in patients harboring *KCNJ10* *gain-of-function* mutations suggests that dysfunction in the astrocytic-dependent K⁺ buffering may be a common mechanism contributing to seizures as well as the core behavioral features of ASD. Kir4.1 *gain-of-function* mutations may lead to faster and larger influx of K⁺ into astrocytes, during intense neuronal activity, resulting in membrane depolarization and higher intracellular Ca²⁺ elevations in these cells. Ca²⁺ elevations in astrocytes are associated with gliotransmitters' release, such as glutamate and D-serine that trigger discharges in neurons and promote local neuronal synchrony and epileptic activity (Steinhäuser et al., 2015). Abnormal synaptic function is the common basis for ASDs. Inasmuch as the activity of many thousands of synapses is controlled by a single astrocyte and this cell type makes up 90% of all human brain cells, defective astrocyte-dependent CNS development and regulation of K⁺ homeostasis in the brain represent original mechanistic hypotheses linking the allelic variations we have identified in *KCNJ2* and *KCNJ10* and ASD.

ChannelASD-CHANNELEPSY PHENOTYPE

Mutations in K⁺ channels have been widely associated with several forms of channelepsies (D'Adamo et al., 2013). The analysis of the literature performed here shows that in nearly all cases affected by K⁺ channelASDs, epilepsy is always comorbid. The degree of clinical and genetic overlap between K⁺ channelASDs and some K⁺ channelepsies, suggests that a subcategory named *channelASD-channelepsy phenotype* may be distinguished. Indeed, this scenario could be envisioned for some other channel types, playing significant roles in brain development, behavior and cognition. An

effective example in this direction comes from Dravet's syndrome (DS), a devastating neurodevelopmental disorder (Catterall, 2000; De Jonghe, 2011). DS is characterized by comorbidity of epilepsy and psychiatric disorders, and linked to Nav1.1 (*SCN1A*) channel dysfunction. Indeed, together with early-life intractable seizures, patients develop devastating psychomotor, cognitive and behavioral deficits persisting through adulthood (Bender et al., 2012). Thus, *SCN1A* has been suggested as a candidate gene for ASD (Weiss et al., 2003). The implication of this gene was confirmed by whole-exome sequencing of sporadic ASD cases that revealed a missense mutation in a severely affected patient with evidence of early onset, language delay, epilepsy, and mild intellectual disability. This mutation leads to replacement of the highly conserved proline 1894 with lysine, and is predicted to be functionally deleterious (O'Roak et al., 2011). *Scn1a*^{-/+} rodents recapitulate DS (Yu et al., 2006; Kalume et al., 2007). The loss of Nav1.1 channels, which is mostly expressed in GABAergic interneurons, leads to a marked reduction in the inhibitory activity of these cells that reverberates onto prefrontal and hippocampal pyramidal neurons, which consequently fire at higher frequencies (Han et al., 2012). Remarkably, these mice show epilepsy and a spectrum of behavioral abnormalities characteristic of ASD such as hyperactivity, anxiety, altered behavioral interaction and deficits in context-dependent spatial memory (Yu et al., 2006; Han et al., 2012). Thus, it could be possible to hypothesize that distinct channel-dependent autism/epilepsy cases share a common etiopathological denominator, namely an excitatory-inhibitory imbalance generating a non-permissive substrate for the physiological development of the proper cognitive and behavioral skills, resulting in *channelASD-channelepsy phenotype*.

CONCLUDING REMARKS

Autism spectrum disorders are amongst the most common neuropsychiatric diseases (Blumberg et al., 2013) that show increasing prevalence over the past years and no discrimination in terms of ethnicity, family income or educational levels. However, how much of this increase is due to a broader definition of ASD and better efforts in diagnosis or an effective increased incidence of the disorders, is unclear. The number of ASDs cases that could be accounted for a channel-dependent pathogenesis and classified in the *channelASD* subgroup, remains to be established. Inasmuch as ~ 70 million people suffer from ASD and nearly 500 ion channel proteins are encoded by the human genome, the worldwide number of *channelASD* cases might not be negligible.

Despite the efforts made, the etiology of ASD remains largely elusive because many cases arise from a mixture of multiple environmental and genetic factors. In addition, the modalities by which the combinations of different genetic variations contribute to the overall risk to develop the disease are mostly obscure. Nevertheless, a mounting body of evidence indicates that ion channel dysfunction may well enhance autism susceptibility (Schmunk and Gargus, 2013) also when other contributing alleles are co-inherited. Direct and indirect defects in K⁺ channels have been implicated in ASDs pathogenesis, likely altering crucial neural network processes in several brain areas including the cerebellum, a structure that emerges as critically involved in determining the core features of ASDs. Abnormal synaptic transmission and

dendritic spine pathology play crucial roles in ASDs. Notably, the activity of many thousands of synapses is controlled by a single astrocyte. Thus, aberrant astrocyte-dependent synaptic functions and CNS development, induced by defective ion channels, represent an interesting causative hypothesis for ASDs (D'Adamo et al., 2011a; Sicca et al., 2011; Ambrosini et al., 2014). Undeniably, the high heterogeneity of ASDs makes the solution of the “autism puzzle” an extremely difficult task. Notwithstanding, to ensure the best possible outcomes for children affected by this devastating disease, comprehensive socio-economic policies and coordinated scientific efforts are required as a matter of urgency.

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